Report for 2001NJ941B: A Continuation Proposal: Factors controlling methylmercury degradation in Pine Barrens lakes and the Meadowlands

There are no reported publications resulting from this project.

Report Follows:

Factors controlling methylmercury degradation in Pine Barrens lakes and the Meadowlands

Problem and Research Objectives: Mercury concentrations in fish of the Pine Barrens lakes are elevated as a consequence of the atmospheric deposition of mercury (Hg) and in-lake methylation processes (NJ DEP, 1994;Ruppel *et al.*, 1994). A range of tissue concentrations of Hg in fish collected from different lakes suggests that the production of methylmercury (MM) is affected by factors that are unique to each lake. Recent work (Pak and Bartha, 1998) showed similar production rates of MM in the sediments of three Pine Barrens lakes suggesting that transport to, and/or MM degradation in, the water column, may be responsible for between-lakes variability. Availability of MM to the aquatic food chain might be controlled by degradation (and possibly production) of MM in the water column following the flux of MM from sediments. Our research addresses the degradation of MM in water samples and how it is affected by the physical-chemical and biological parameters in Pine Barrens lakes. Samples collected at the Meadowlands, a highly contaminated site where low MM/total Hg (Hg_T) concentration ratios in the water were detected, are included for the sake of comparison.

Two objectives address the hypothesis that MM production is controlled by its degradation in the water column:

(i) To relate the MM concentration and rate of its degradation to the MM/Hg_T ratio.
(ii) To determine if the abundance and expression of bacterial mercury resistance (*mer*) genes in the microbial communities of the lakes control MM production by stimulating its degradation. Enzymes encoded by these genes are known to degrade MM to volatile Hg(0).

Procedures and Methods

Mercury analyses: Total Hg is measured water samples (200 - 500 ml) in Dr. Reinelder's lab by the cold vapor atomic fluorescence spectrometry (CVAFS) technique (Bloom and Fitzgerald, 1988) using a Tekran 2500 CVAFS mercury detector. Dr. Reinfelder is currently setting up his lab for MM analysis. Data for MM concentrations reported here was obtained through a contract with Flett Laboratories, Inc., (Winnipeg, Manitoba).

Physical-chemical parameters: Hand held probes are used to measure pH, temperature, salinity, dissolved O_2, and conductivity in the field during sample collection. Total organic carbon is measured at the laboratory of Dr. Seitzinger at IMCS.

Microbiological and molecular parameters: Routine protocols are used for the enumeration of heterotrophic bacteria and assessment of community diversity (Barkay, 1987). The presence of *mer* genes and their expression are detected by protocols developed in our laboratory (Nazaret *et al.*, 1994). We are currently developing quantitative approaches for gene and transcript detection using a recently purchased quantitative PCR instrumentation.

Principle findings and significance

Samples were collected during Aug. 2000 and Apr. 2001from four Pine Barrens Lakes and from four sites in Berry's Creak in the Meadowlands. The most significant findings are summarized below:

I. An inverse relationship between the proportion of MM in total Hg (Hg_T) and Hg_T concentration has emerged from data collected to date (Fig. 1). It resulted from a large discrepancy in Hg_T concentrations (μg/L for Meadowlands and ng/L for Pine Barrens) with similar MM concentrations (ng/L in both study sites). Others have demonstrated this trend with samples collected from numerous environments. To the best of our knowledge, our hypothesis regarding the role of inducible microbial transformations in MM degradation, is, at present, the only plausible explanation for this "paradox".

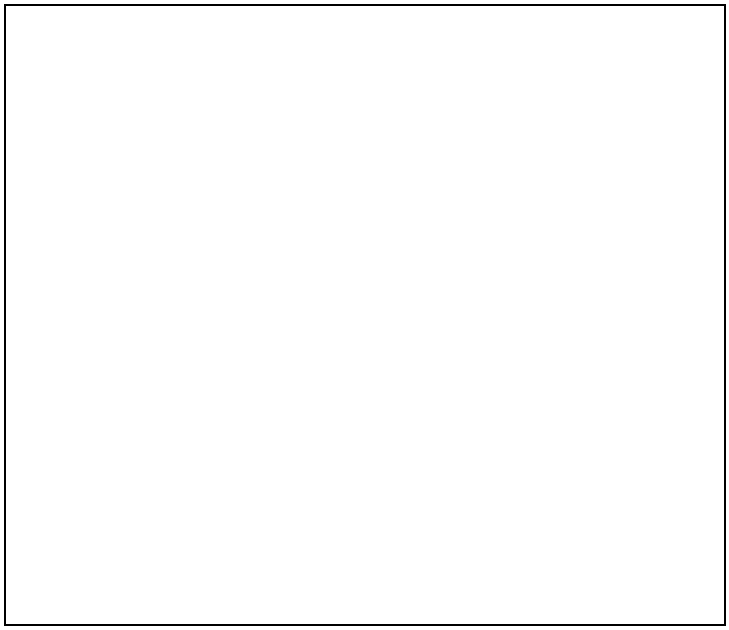

Fig. 1: The relationship between the percent MM in Hg_T and Hg_T concentrations in Pine Barrens samples collected in Aug. 00 (open circles) and in Apr. 01 (open triangles), and in Meadowlands samples collected in Aug. 00 (full squares) and Apr. 01 (full diamonds).

II. Initial analysis of the response of microbial communities to Hg suggests that in the highly contaminated water of Berry's Creek (Meadowlands) the communities are adapted to Hg stress while Pine Barrens communities are not (Table 1). This conclusion is based on: (i) there was a higher tolerance to Hg(II) among heterotrophic bacteria from Meadowlands samples, (ii) the diversity of the resistant bacteria was higher in Meadowlands communities than in Pine Barrens communities, and (iii) *merA* genes specifying the reduction of Hg(II) to Hg(0) were detected in biomass from the Meadowlands but not in biomass from the Pine Barrens (Fig. 2). Physical-chemical measurements showed that the Pine Barrens waters were acidic (pH 4 to 5), had low conductivity (< 50 μS/cm), and no measurable salinity while Meadowlands waters had a neutral pH, conductivity of about 900 μS/cm, and were slightly saline at 0.4 parts per thousand. Remaining parameters, TOC (0.5 – 1 mM) and dissolved O_2 (6.5 – 7 mg/L), were similar for Meadowlands and Pine Barrens samples.

Table 1: Microbiological parameters describing the response of the microbial communities in the Meadowlands and Pine Barrens Lakes to Hg (Aug. 00 sampling).

	Meadowlands sites:			**Pine Barrens sites:**	
Parameter	**MLU**	**MLM**	**MLD**	**PBH**	**PBB**
Total heterotrophs (CFU/ml)	NA[1]	$4.8x10^4$	$2.2x10^4$	NA	$1.6x10^3$
Hg^R heterotrophs (CFU/ml)	$2.8x10^3$	$4.2x10^3$	$1.8x10^3$	NA	
Diversity – heterotrophic community (H')[2]	NA	2.845	2.770	NA	2.659
Diversity – Hg^R community (H')	2.714	2.067	2.311	NA	0.000

[1]NA: Not available – Number of colonies observed was too low to allow determinations.

[2]Shannon-Weaver diversity index, calculated as:

$$H' = -\sum^{S^*}_{i=1} (P_i \ln P_i)$$

Where: P_i = No. of colonies in a specific morphology group / Total No. of colonies analyzed

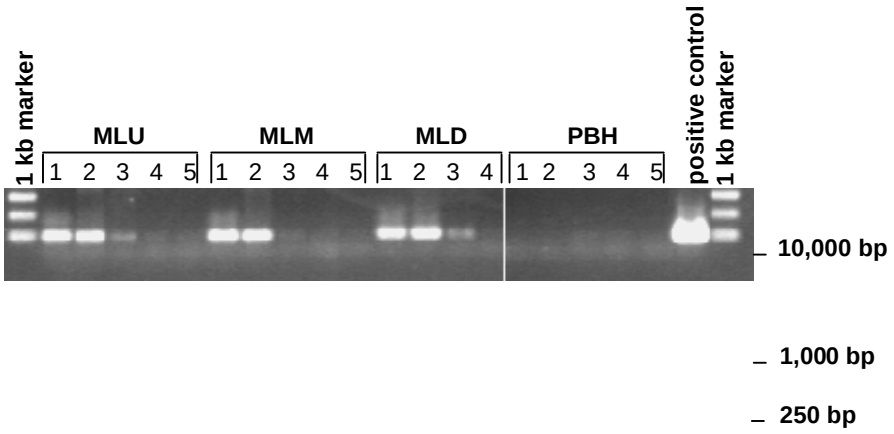

Fig. 2: Results of PCR amplifications using primers targeting highly conserved sequences in *merA*. Ten fold dilutions (dilution factors of 10^1 to 10^5, lanes marked as 1 through 5, respectively) of DNA extracted from the microbial biomass in Meadowlands water (MLU, MLM, and MLD) and in Harrisville Lake (PBH) were amplified and amplification products were separated on 1% agarose gel. Positive control: amplification of DNA from a plasmid carrying a known *merA* gene.

Summary and future directions

Our results show that (i) proportionally more MM accumulates in Pine Barrens Lakes where Hg_T, mostly from atmospheric deposition is at the ng/L concentrations than in Meadowlands waters, and (ii) low population densities of bacteria with the potential to degrade MM were detected in Pine Barrens Lakes water. While these findings provide a tentative support to the hypothesis that in the Pine Barrens active microbial MM degradation is not induced due to low concentrations of inorganic Hg, further and more rigorous support is needed. This support will be obtained by, (i) measuring the rate of ^{14}C-MM degradation, (ii) quantitating *mer* genes and their expression in samples collected at Pine Barrens Lakes and the Meadowlands. The complete sets of data for all microbiological, molecular and physical-chemical parameters will be statistically analyzed to determine which of the measured parameters most significantly affect MM production.

Literature cited

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